A

```
1     MKFLGGNDDRNGRGGVGVGTDAIVGSRGGVSQDAADAAGAAAAAAVGYVF
51    QQRPSPGGVGVGVGGVGGGVPGVGAVGSTLHEAAAAEYAAHFAQKQQQTR
101   WACGDDGHGIDNPDKWKYNPPMNPANAAPGGPPGNGSNGGPGAIGTIGMG
151   SGLGGGGGGGAGGGNNGGSGTNGGLHHQSMAAAAANMAAMQQAAALAKHN
201   HMISQAAAAVAAQQQHQHPHQQHPQQQQQQQQQAQNQGHPHHLMGGGNGLG
251   NGNGLGIQHPGQQQQQQQQQQQQQQHPGQYNANLLNHAAALGHMSSYAQSG
301   GSMYDHHGGAMHPGMNGGMPKQQPLGPPGAGGPQDYVYMGGQTTVPMGAA
351   MMPPQNQYMNSAAVAAANRNAAITTSTAKKLWEKSDGKGVSSSTPGGPLH
401   PLQIPGIGDPSSVWKDHTWSTQGENILVPPPSRAYAHGGASDTSNSGNAG
451   ILSPRDSTCAKVVEYVFSGSPTNKDSSLSGLEPHLRNLKFDDNDKSRDDK
501   EKANSPFDTNGLKKDDQVTNSNGVVNGIDDDKGFNRTPGSRQPSPAEESQ
551   PRPPNLLFPPLPFNHMLMDHGQGMGGGLGGVVGSGNGVGGGSGGGGAGGA
601   YAAHQQMAAQMSQLQPPMMNGVGGGMPMAAQSPMLNHQAAGPNHMESPGN
651   LLQQQNFDVQQLFRSQNPGLAAVATNAAAAAAAAAAATSAASAAAAVGAP
701   PVPNGSLQQSQQQQQQQQQQQQQQQQQMHMAAASQQFLAAQQQAQNAAYAAQ
751   QATSYVINPGQEAAPYMGMIAAAQMPYYGVAPWGMYPGNLIPQQGTQPRR
801   PLTPSQQGAENQPYQVIPAFLDHTGSLLMGGPRTGTPMRLVSPAPVLVPP
851   GATRAGPPPPQGPQLYQPQPQTAQQNLYSQQNGSSVGGLALNTSSLTGRR
901   DSFDRSTSAFSPSTMDYTSSGVAAAANAVNSTVAQAAAAAAAAAARGKW
951   PGAMSGAASGAYGALGAGNASASPLGAPITPPPSAQSCLLGSRAPGAESR
1001  QRQQQQQQLAAVGLPATAAAAQAAVAAAANNMFGSNSSIFSNPLAIPGTA
1051  AVAAAAAAAAAANSRQVAATAAAAAAVAAAAGGVGGAPQPGRSRLLEDFR
1101  NQRYPNLQLRDLANHIVFFSQDQHGSRFIQQKLERATAAEKQMVFSEILA
1151  AAYSLMTDVFGNYVIQKFFEFGTPEQKNTLGMQVKGHVLQLALQMYGCRV
1201  IQKALESISPEQQQEIVHELDGHVLKCVKDQNGNHVVQKCIECVDPVALQ
1251  FIINAFKGQVYSLSTHPYGCRVIQRILEHCTAEQTTPILDELHEHTEQLI
1301  QDQYGNYVIQHVLEHGKQEDKSILINSVRGKVLVLSQHKFASNVVEKCVT
1351  HATRGERTGLIDEVCTFNDNALHVMMKDQYANYVVQKMIDVSEPTQLKKL
1401  MTKIRKNMAALRKYTYGKHINAKLEKYYMKITNPITVGTGAGGVPAASSA
1451  AAVSSGATSASVTACTSGSSTTTTSTTNSLASPTICSVQENGSAMVVEPS
1501  SPDASESSSSVVSGAVNSSLGPIGPPTNGNVVL
```

B

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1     MADPNWAYAPPTNYYADHSIAKPIMISGGHPSQDQGHSPKSESFGQSVTT
51    AFNGMVDNLVGSPSSSVQQRNYFTTTPFPISRSPNDRNDDKIMGNGSYGV
101   PIPIPQDGVPQGTPDFQMTPFLQQGGHLIGGSPNGPVQVSGNWYSGGAGI
151   FSTMQQADPSNGMPGMAAEFVNNENGMPGPNGMHQQAMISGSPPFPYQNM
201   MNLTTSFGAMGLGPQQIQQRDPQMFQQPILHEPIQGMAQNGFGQQVFFTQ
251   MQNQQHPQGQAQQQLQQLAQQHQQQQNSQQFFGQGPNGMGNGGVMNDWSQ
301   RSFGMPQQQAQQNGLPPNFSQNPPRRRGPEDPNGQTPKTLQDIKNNVIEF
351   AKDQHGSRFIQQKLERASLRDKAAIFTPVLENAEELMTDVFGNYVIQKFF
401   EFGNNEQRNQLVGTIRGNVMKLALQMYGCRVIQKALEYVEEKYQHEILGE
451   MEGQVLKCVKDQNGNHVIQKVIERVEPERLQFIIDAFTKNNSDNVYTLSV
501   HPYGCRVIQRVLEYCNEEQKQPVLDALQIHLKQLVLDQYGNYVIQHVIEH
551   GSPSDKEQIVQDVISDDLLKFAQHKFASNVIEKCLTFGGHAERNLIIDKV
601   CGDPNDPSPPLLQMMKDPFANYVVQKMLDVADPQHRKKITLTIKPHIATL
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Figure S1

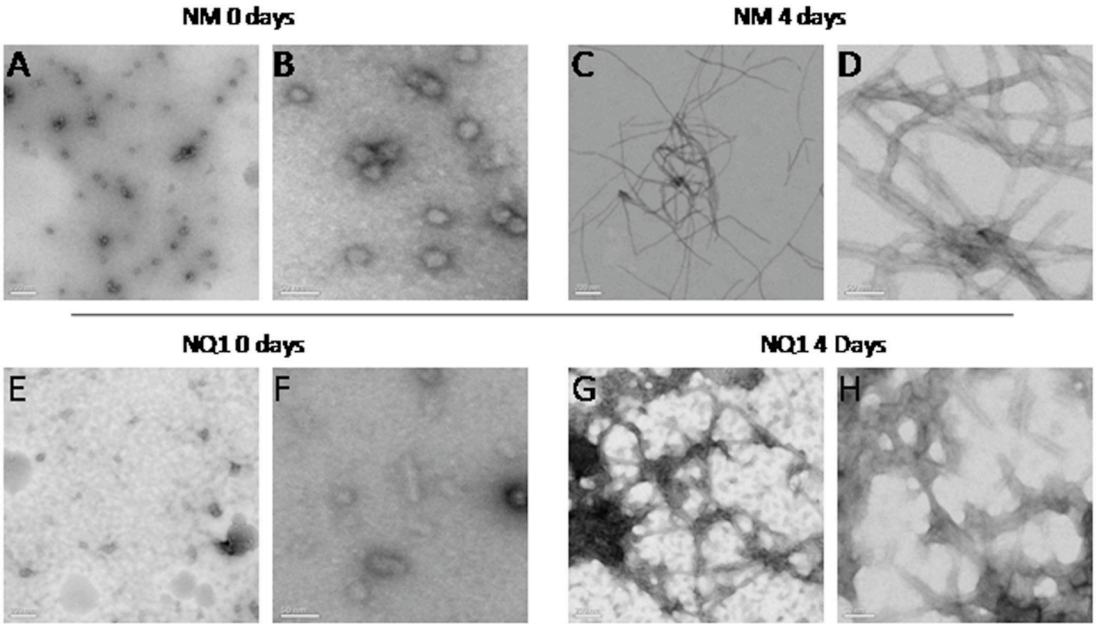

Figure S2

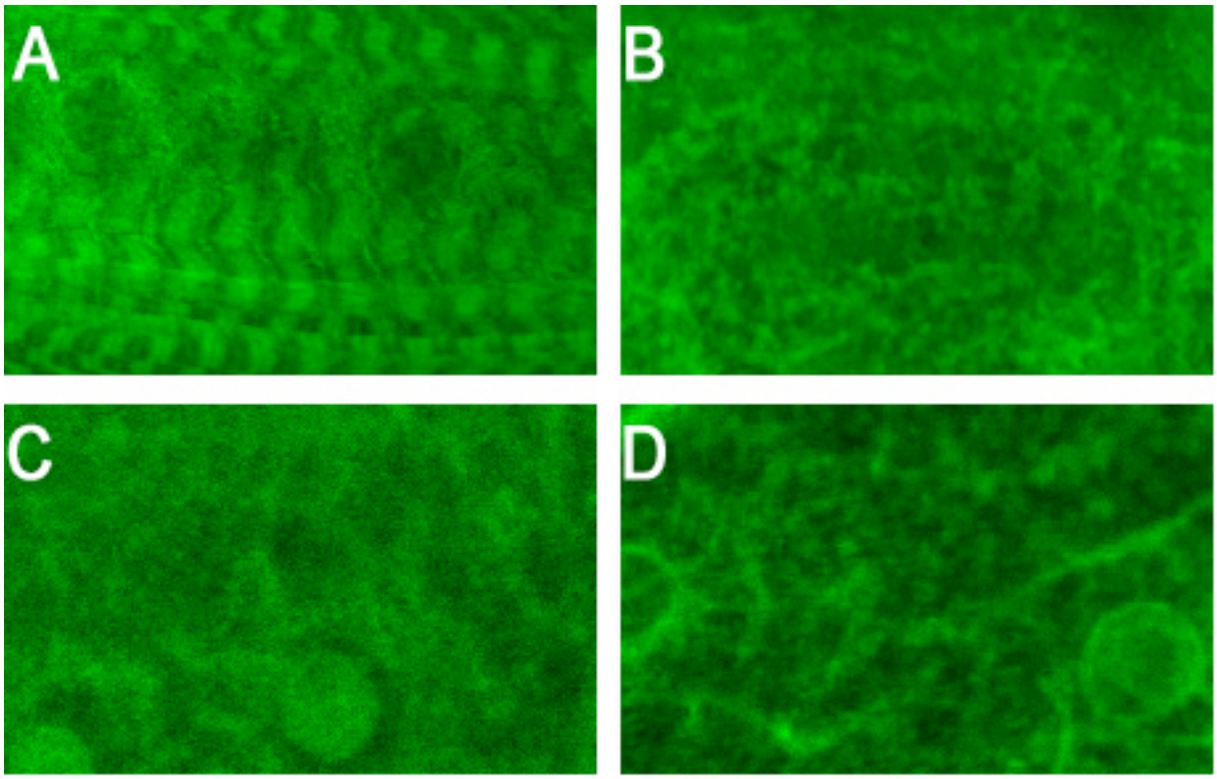

Figure S3

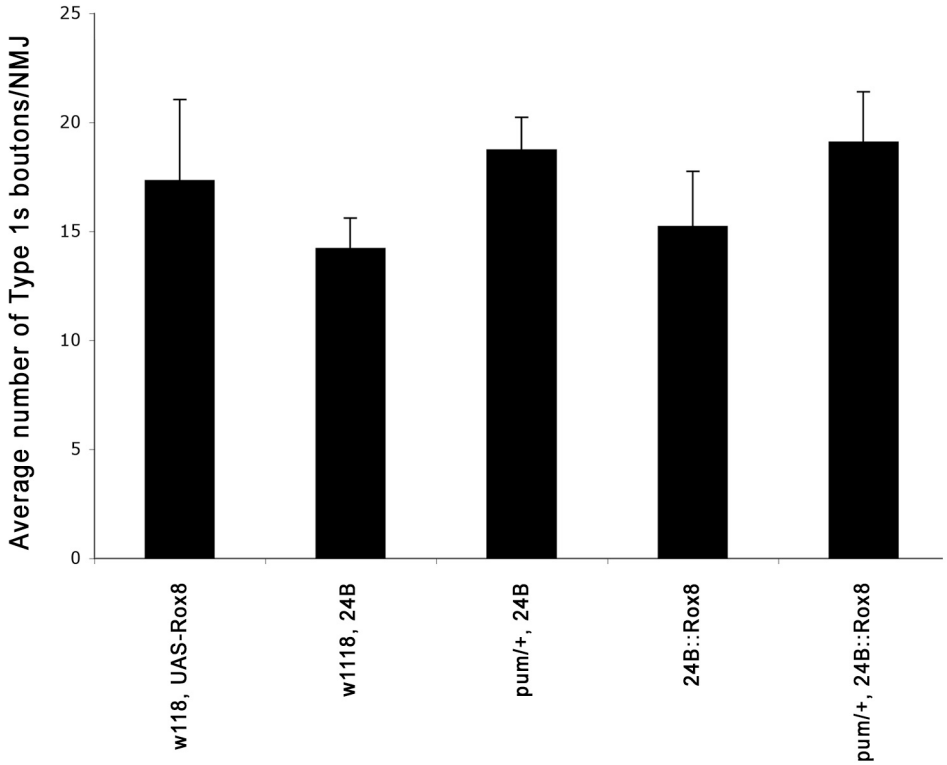
Average number of Type 1s boutons/NMJ
25
20
15
10
5
0
w118, UAS-Rox8
w1118, 24B
pum/+, 24B
24B::Rox8
pum/+, 24B::Rox8

Figure S4

Supplementary Figure Legends

Figure S1. A, *Drosophila melanogaster* Pumilio amino acid sequence with Q/Ns highlighted. Domains underlined as shown: NQ1 Domain, NQ2 Domain, RNA Binding Domain. **B,** *Caenorhabditis elegans* PUF-9 amino acid sequence with Q/Ns highlighted.

Figure S2. EM images of unconverted (0 days) and converted (4 days) Pum NQ1 and Sup35NM proteins. The scale bar in the left panel of each pair is 200 nm, and the bar in the right panel of each pair is 50 nm. Note the absence of fibrils in the 0 day images.

Figure S3. Confocal images of *Drosophila* larvae muscle 12 using anti-GFP to show NQ1-CFP expression when driven with 24B. A, *UAS-NQ1/24B-GAL4*. Note stereotyped muscle pattern with no visible aggregates. **B**, Higher magnification of muscle seen in A. **C**, *UAS-tauGFP/24B-GAL4* stained with anti-GFP. **D**, *UAS-mCD8GFP/24B-GAL4* stained with anti-GFP. C and D are controls.

Figure S4. Bar graph of 1s bouton numbers when Rox8 is expressed postsynaptically. Genotypes indicated below the *x*-axis; full genotypes and numbers of A2 and A3 hemisegments scored are: *UAS-Rox8/+* (n=9); *24B-GAL4/+*(n=34); pum^{ET9}*, 24B-GAL4/+* (n=34); *UAS-Rox8/24B-GAL4* (n=28); *UAS-*

Rox8/pum^{ET9}*, 24B-GAL4* (n=32). Error bars are shown. No differences in bar height are significant.